DATA EVALUATION RECORD

CAPHRA/β-CYFLUTHRIN

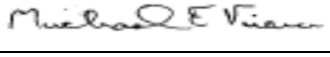
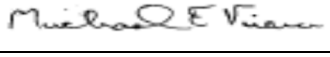
Study Type: OCSPP Non-Guideline; *In Vitro* Metabolism Kinetics

EPA Contract No. EP-W-16-018
Task Assignment No.: 32-3-034 (MRID 50803903)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

3201 Jermantown Rd., Suite 400
Fairfax, VA 22030

Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/17/2019
Secondary Reviewer: Michael E. Viana, Ph.D., DABT	Signature: Date:	 05/23/2019
Quality Assurance: Sarah E. Saucier, Ph.D.	Signature: Date:	 05/30/2019
Project Manager: Michael E. Viana, Ph.D., DABT	Signature: Date:	 05/31/2019

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel**.** Contractor's role did not include establishing Agency policy.

EPA Reviewer: Evisabel Craig, Ph.D., DABT **Signature:** E.C. Craig
Risk Assessment Branch VI, HED (7509C) **Date:** 8/1/2019
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: OCSPP Non-guideline; *In Vitro* Metabolism Kinetics.

PC CODE: 118831

DP BARCODE: D451266

TXR#: 0057896

TEST MATERIAL (PURITY): β-Cyfluthrin (99% a.i.)

SYNONYMS: (*R*)-cyano(4-fluoro-3-phenoxyphenyl)methyl (1*S*)-*rel*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

CITATION: Brown, S. (2019) β-Cyfluthrin: A study to determine the kinetics of metabolism of β-cyfluthrin in selected expressed human carboxylesterase (CES) and cytochrome P450 (CYP) enzymes; final report. Concept Life Sciences Dundee, Dundee Technopole, Dundee, United Kingdom. Laboratory Project ID: CXR1723-III β-cyfluthrin, January 10, 2019. MRID 50803903. Unpublished.

SPONSOR: Council for Advancement of Pyrethroid Human Risk Assessment, LLC (CAPHRA), c/o Household and Commercial Products Association, 1667 K Street, NW, Suite 300, Washington DC

EXECUTIVE SUMMARY: In a non-guideline, *in vitro* metabolism study (MRID 50803903), the apparent intrinsic clearance (CL_{int}) of β-cyfluthrin (99% a.i.; Lot # 0507200901) was determined in recombinant microsomes. The enzymes expressed by these systems were CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C19, CYP2D6*1, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CES1b, and CES2 (see Appendix 1 at the end of this DER for product details); a control system also was included. For the CYP enzymes, preliminary experiments were conducted with β-cyfluthrin to screen for metabolism by the enzyme variants. Duplicate incubations were conducted with the CYP enzymes (10 pmol/mL) and β-cyfluthrin (0.1 µM) with a minimum protein concentration of 0.1 mg/mL achieved by the addition of control microsomes. Incubations with control microsomes at the same concentrations as the CYP microsomes were performed to correct for endogenous CES activity. If the total rate (k_{dep}) for the CYP enzyme was greater than twice the rate of the control microsomes, it was selected for further experimentation. For the CES enzymes, a preliminary experiment was conducted with duplicate incubations of β-cyfluthrin (0.1 µM) with CES1b at 2, 5, and 10 pmol/mL and CES2 at 20, 50, and 100 pmol/mL with bovine serum albumin (BSA) to achieve a minimum protein concentration of 0.1 mg/mL. Control incubations also were performed with BSA alone to account for non-enzymatic loss of β-cyfluthrin. The main study was conducted in duplicate with CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, CYP3A5, CES1b, and CES2 in the presence of NADPH (CYP only) with 0.1 µM β-cyfluthrin. Two experiments

were conducted with the data from the first experiment used for selection of time points used in the second experiment (a third confirmatory experiment was conducted for CYP2C8). The final concentrations of β-cyfluthrin were determined by LC/MS.

Rates of depletion were determined from plots of the natural logarithm of the percentage of β-cyfluthrin remaining against incubation time by linear regression. Estimates of CL_{int} for CYP and CES enzymes were calculated with the following equation:

$$CL_{int} = \frac{0.693}{t½} \times \frac{\text{Incubation volume (mL)}}{\text{pmol CYP or CES}}$$

RESULTS

PRELIMINARY EXPERIMENTS: Duplicate incubations with 0.1 µM β-cyfluthrin in recombinant human microsomes were conducted in the presence of NADPH; incubation times were not reported. Preliminary results for the CYP enzymes were presented in Table 1 on page 19 of MRID 50803903 and are included in Appendix 2 at the end of this DER. CYP2C8, CYP2C9*1, CYP2C19, CYP3A4, and CYP3A5 metabolized β-cyfluthrin at ≥1.75× the rate of control microsomes and were selected for the main study experiments. The increases for CYP3A4 and CYP3A5 did not achieve the two-fold criterion level but were selected regardless. Preliminary results for the CES enzymes were presented in Table 2 on page 20 of MRID 50803903 and are included in Appendix 2 at the end of this DER. Concentrations of 2 pmol/mL and 50 pmol/mL were selected for CES1b and CES2, respectively, for the main study.

MAIN STUDY EXPERIMENTS: Results for the main study experiments were presented in Tables 3 (CYP) and 4 (CES) on pages 21 and 22, respectively, of MRID 50803903 and are included in Appendix 3 at the end of this DER. Because there was a 1.7-fold difference in the calculated CL_{int} values for metabolism with CYP2C8 between the experiments carried out on April 26 and May 1, a further experiment was conducted. The data from the third experiment (June 5) were similar to the data from May 1; therefore, the data from April 26 were not used in the calculation of the mean values. Estimated CL_{int} values ranged from 1.55 µL/min/pmol for CYP2C9*1 to 8.06 µL/min/pmol for CYP2C19. β-cyfluthrin was metabolized to a lesser extent by CES1b and CES2 with estimated CL_{int} values of 1.27 µL/min/pmol and 0.0582 µL/min/pmol, respectively.

REVIEWER'S COMMENTS: This is a non-guideline study and was submitted as part of CAPHRA's effort to assess the pharmacokinetic properties of the pyrethroids.

APPENDIX 1

Source of test systems: Recombinant human "supersomes" were purchased from Corning B.V. Life Sciences, Amsterdam, The Netherlands. These "supersomes" are microsomes having recombinant human enzymes expressed by baculovirus-infected insect cells (see table below).

Expressed enzyme	Product number	Lot Number	Expressed enzyme	Product number	Lot Number
CYP1A2	456203	7320001	CYP3A4	456202	5224002
CYP2A6	456254	5315001	CYP3A5	456256	5258001
CYP2B6	456255	5239002	CYP3A7	456237	5246004
CYP2C8	456252	7255002	CYP4A11	456221	5266001
CYP2C9*1	456258	6257001	CES1b	453320	6230004
CYP2C19	456259	7262001	CES2	453322	6084004
CYP2D6*1	456217	456217	Control	456244	6180001
CYP2E1	456206	5265003			

(copied from page 11 of MRID 50803903)

APPENDIX 2

Expressed enzyme	Date	β-Cyfluthrin Conc µM	Total K_{dep} min^{-1}	Control K_{dep} min^{-1}
CYP2C19	19-Apr-18	0.1	0.146	0.00914
CYP2C8	19-Apr-18	0.1	0.0317	0.00912
CYP2C9*1	19-Apr-18	0.1	0.0315	0.00912
CYP3A4	20-Apr-18	0.1	0.0217	0.0110
CYP3A5	20-Apr-18	0.1	0.0203	0.0116
CYP2D6	20-Apr-18	0.1	0.0114	0.0110
CYP4A11	20-Apr-18	0.1	0.0112	0.0103
CYP2B6	19-Apr-18	0.1	0.0107	0.00912
CYP2E1	20-Apr-18	0.1	0.00951	0.0110
CYP2A6	19-Apr-18	0.1	0.00893	0.00912
CYP3A7	20-Apr-18	0.1	0.00732	0.0110
CYP1A2	19-Apr-18	0.1	0.00557	0.00912

(copied from page 19 of MRID 50803903)

Expressed enzyme	Date	Cyfluthrin conc µM	CES K_{dep} min^{-1}
CES1b 2 pmol/mL	30-Apr-18	0.1	0.00898
CES1b 5 pmol/mL	30-Apr-18	0.1	0.00456
CES1b 10 pmol/mL	30-Apr-18	0.1	0.0114
CES2 20 pmol/mL	30-Apr-18	0.1	0.00140
CES2 50 pmol/mL	30-Apr-18	0.1	0.00216
CES2 100 pmol/mL	30-Apr-18	0.1	0.00152

(copied from page 20 of MRID 50803903)

APPENDIX 3

Expressed Enzyme	Date	β-Cyfluthrin Conc µM	kdep min^{-1}	$t_{1/2}$ min	CL_{int} µL/min/pmol CYP	Mean CL_{int} µL/min/pmol CYP
CYP2C19	26-Apr-18	0.1	0.0625	11.1	6.25	8.06
	01-May-18	0.1	0.0987	7.0	9.87	
[1]CYP2C8	26-Apr-18	0.1	0.0143	48.5	1.43	2.45
	01-May-18	0.1	0.0245	28.4	2.45	
	05-Jun-18	0.1	0.0245	28.3	2.45	
CYP3A4	26-Apr-18	0.1	0.0151	45.8	1.51	1.92
	01-May-18	0.1	0.0233	29.7	2.33	
CYP3A5	26-Apr-18	0.1	0.0189	36.7	1.89	1.81
	01-May-18	0.1	0.0174	39.9	1.74	
CYP2C9*1	26-Apr-18	0.1	0.0147	47.1	1.47	1.55
	01-May-18	0.1	0.0163	42.7	1.63	

[1] Data from the CYP2C8 experiment on 26 April 2018 should not be used, and have not been included in the calculation of means, as there is a notable difference in calculated CL_{int} between this experiment and those of 01 May 2018 and 05 June 2018.

Rates of β-cyfluthrin depletion in control supersomes were subtracted from the total rate to correct for endogenous CES activity.

(copied from page 21 of MRID 50803903)

Expressed Enzyme	Date	β-cyfluthrin Conc µM	kdep min^{-1}	$t_{1/2}$ min	CL_{int} µL/min/pmol CES	Mean CL_{int} µL/min/pmol CES
CES1b 2 pmol/mL	28-May-18	0.1	0.00185	374.9	0.925	1.27
	30-May-18	0.1	0.00323	214.8	1.61	
CES2 50 pmol/mL	28-May-18	0.1	0.00210	329.9	0.0420	0.0582
	30-May-18	0.1	0.00372	186.6	0.0743	

Rates of β-cyfluthrin depletion in BSA were subtracted from the total rate to correct for non specific loss of β-cyfluthrin.

(copied from page 22 of MRID 50803903)